December 1968 Brief 68-10554

NASA TECH BRIEF

A Microlagoon Technique for the Culture of Mammalian Cells

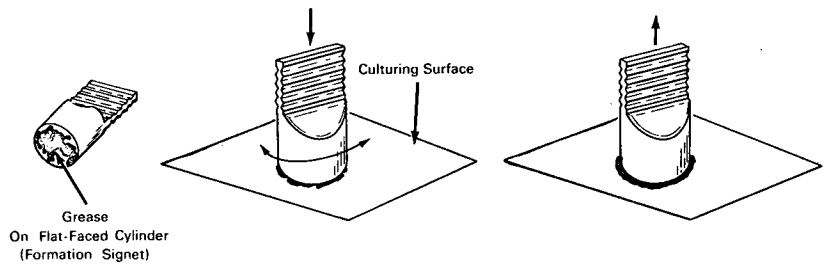

FIGURE 1

FIGURE 2

In many cytological investigations it is either desirable or necessary to deal with individual cells or with small groups of cells in monolayer culture. A typical example is the development of a microclone from a single parent cell. Since it is very difficult to maintain small numbers of cells in culture, it is usually necessary in such cases to begin with a well-populated monolayer and to select test specimens from those areas of the field having a sufficiently sparse density of cells. This procedure has serious drawbacks, however, in that the continuous migration and mitoses of cells within the test area make long-term observation and identification of particular experimental specimens difficult, if not impossible. For such investigations it is obviously desirable to have some means for physically partitioning a large field of cells into microregions without restricting the free access of the common culture medium to all parts of the field.

An effective technique has been developed for obtaining such micropartitioning in a simple and reproducible manner by forming a field of tiny ponds or lagoons on the surface of a suitable culturing vessel. The basic technique (Figure 1) consists in applying under sterile conditions a thin coating of a suitable nontoxic grease to the smooth end of a small solid cylinder, by using a fine brush or by simply coating the cylinder end directly from a small glass syringe. The coated end is pressed firmly against the surface of the desired culturing vessel (for example, a Petri dish or perfusion chamber cover glass) and oscillated slightly to obtain a uniform contact of the grease with the surface. The cylinder, which is termed the "formation signet," is then lifted rapidly from the surface by applying a strong force in the direction of the cylinder axis. Due to the cohesion of the grease, this separation results in the grease being pulled into a

(continued overleaf)

fine meshwork of connected ridges. Very small holes are left in the thin grease layers of many of the flat areas enclosed by the ridge network, and the grease sheet surrounding these holes contracts immediately after formation; thus, the openings are enlarged and the free surface area is exposed. This overall action produces a field containing large numbers of small lagoons in which the walls consist of the grease material and the bottoms consist of free culturing surface.

Within any one field (equal in extent to the area of the formation signet face), a wide range of lagoon shapes and sizes is usually found. Typical lagoon formations obtained in this manner are illustrated in Figure 2. The lagoons so formed are subsequently inoculated with cells for test purposes. The size of the depressions within the initial ridge network, and hence the size distribution of the resulting lagoons, is largely governed by the rapidity with which the signet is separated from the vessel surface during formation and by the parallel alinement of the signet face and the vessel surface at separation. The greased signet used in preparing the lagoon field adheres very firmly to either plastic or glass upon pressing, and a firm grip is required to lift it normally from the surface with the desired rapidity. A series of signets have been specially designed for specific types of lagoon field formation.

Note:

Documentation for the innovation is available from:

Clearinghouse for Federal Scientific and Technical Information
Springfield, Virginia 22151
Price $3.00
Reference: B68-10554

Patent status:

No patent action is contemplated by NASA.

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